

with the requirements of 37 CFR 1.821(a)(1) and 1.821 (a) (2). In addition, Pages 7, 14, 15 and 74 are amended herein to correct obvious typographical errors. No new matter is added.

The Office Action appears to allege that sequences without proper sequence identifiers are disclosed in Figures 1A, 1B, 1C, 1D, 10A, 10B, 10C, 11 and 13. However, all sequences in the above mentioned figure legends are found in the sequence listing previously submitted on December 5, 2001. Thus, applicants believe that submission of a substitute sequence listing is not required. However, appropriate sequence identifiers were missing from the corresponding brief descriptions for these figures. This amendment is submitted to include appropriate references to the sequence identifiers in the brief descriptions of the figures.


CONCLUSION

If any matters remain to be addressed before substantive examination, the examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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By



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**Marked-up Version of Amended Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)**

In the Specification:

Please replace the paragraph on page 7, lines 8-19 with the following paragraph:

Fig. 1A shows the nucleotide (SEQ ID NO: 1) and corresponding amino acid (SEQ ID NO: 2) sequences of the prototypical [$\beta 1\alpha 1$] $\beta 1\alpha 1$ cassette without an antigen coding region. Unique NcoI, PstI, and XhoI restriction sites are in **bold**. The end of the $\beta 1$ domain and start of the $\alpha 1$ domain are indicated. Fig. 1B shows the nucleotide (SEQ ID NO: 3) and corresponding amino acid (SEQ ID NO: 4) sequences [sequence] of an in-frame antigenic peptide/linker insertion sequence that can be incorporated into the expression cassette at the insertion site shown in Fig. 1A. This sequence includes the rat MBP-72-89 antigen, a flexible linker with an embedded thrombin cleavage site, and a unique SpeI restriction site that can be used for facile exchange of the antigen coding region. Example 2 below discusses the use of the equivalent peptide from Guinea pig, which has a serine in place of the threonine residue in the MBP-72-89 sequence. Figs. 1C (SEQ ID NO: 5-6) and Fig. 1D (SEQ ID NO: 7-8) show exemplary nucleotide sequences and their corresponding amino acid sequences of NcoI/SpeI fragments that can be inserted into the expression cassette in place of the MBP-72-89 antigen coding region. Fig. 1C includes the MBP-55-69 antigen, Fig. 1D includes the CM-2 antigen.

Please replace the paragraph on page 9, lines 18-21 with the following paragraph:

Figs. 10A-C show[s] the amino acid sequences of exemplary $\beta 1$ and $\alpha 1$ domains of (A) human (DRA and DRB1 0101) (SEQ ID NO: 22), (B) mouse (I-E^K) (SEQ ID NO: 23), and (C) rat (RT1.B) [$\beta 1$ and $\alpha 1$ domains (the)] (SEQ ID NO: 24), respectively. The initiating methionine and glycine sequences in the rat sequence were included in a construct for translation initiation reasons [D].

Please replace the paragraph on page 9, lines 22-23 with the following paragraph:

Fig. 11 shows the amino acid [sequences] sequence (SEQ ID NO: 21) of exemplary $\alpha 1$ and $\alpha 2$ domains derived from human MHC class I B*5301.

Please replace the paragraph on page 10, lines 11-24 with the following paragraph:

Fig. 13 is the nucleotide (SEQ ID NO: 43) and protein (SEQ ID NO: 44) sequence of human HLA-DR2-derived RTL303 [(SEQ ID NO: 43 and 44, respectively)]. RTL303 was derived from sequences encoding the beta-1 and alpha-1 domains of HLA-DR2 (human DRB1*1501/DRA*0101) and sequence encoding the human MBP85-99 peptide. Unique NcoI, SpeI and XhoI restriction sites are in **bold**. The end of the beta-1 domain and start of the alpha-1 domain are indicated by an arrow (\blacktriangledown). RTL303 contains an in-frame peptide/linker insertion encoding the human MBP85-99 peptide (**bold**), a flexible linker with an embedded thrombin cleavage site (23), and a unique SpeI restriction site which can be used for rapidly exchanging the encoded amino-terminal peptide. RTL301 is identical to RTL303 except for a single point mutation resulting in an F150L substitution. Two additional proteins used in this study, RTL300 and RTL302, are "empty" versions of RTL301 and RTL303, respectively. These molecules lack the peptide/linker insertion (residues 16-115). Codon usage for glycines 32, and 51 have been changed from the native sequence for increased levels of protein expression in *E. coli* (G.G. Burrows, unpublished observations).

Please replace the paragraph on page 14, lines 3-17 with the following paragraph:

Fig. 25 is a set of graphs showing IL-10 cytokine production induced by RTL pre-treatment was maintained after stimulation with APC/peptide. T cells showed a reduced ability to proliferate and produce cytokines after anti-CD3 or RTL treatment, and the RTL effect was antigen and MHC specific. IL-10 was induced only by specific RTLs, and IL-10 production was maintained even after restimulation with APC/antigen. T cell clones were cultured at 50,000 cells/well with medium, anti-CD3, or 20 μ M RTLs in triplicate for 48 hours, and washed once with RPMI. After the wash, irradiated (2500 rad) frozen autologous PBMC (150,000/well) plus

peptide-Ag (MBP-85-99 at 10 µg/ml) were added and the cells incubated for 72 hr with ³H-thymidine added for the last 18 hr. Each experiment shown is representative of at least two independent experiments. Bars represent mean ± SEM. For cytokine assays, clones were cultured with 10 µg/ml anti-CD3 or 20 µM RTL303 or RTL311 for 48 hours, followed by washing with RPMI and re-stimulation with irradiated autologous PBMC (2500 rad, T:APC=1:4) plus peptide-Ag (10 [α] µg/ml) for 72 hours. Cytokines (pg/ml) profiles were monitored by immunoassay (ELISA) of supernatants. Each experiment shown is representative of at least three independent experiments. Bars represent mean ± SEM.

Please replace the paragraph on page 15, line 10 with the following paragraph:

SEQ ID NO: 28-31 are the nucleic acid [sequence] sequences of primers used for human [β1α1] β1α1.

Please replace the paragraph on page 74, lines 5 - 27 with the following paragraph:

Peptide-specific T cell clones were selected from peripheral blood mononuclear cells (PBMC) of a multiple sclerosis (MS) patient homozygous for HLA-DRB1*1501 and an MS patient homozygous for HLA-DRB1*07, as determined by standard serological methods and further confirmed by PCR amplification with sequence-specific primers (PCR-SSP) (Olerup et al., 1992). Frequencies of T cells specific for human MBP85-99 and CABL were determined by limiting dilution assay (LDA). PBMC were prepared by ficoll gradient centrifugation and cultured with 10 µg/ml of either MBP85-99 or CABL peptide at 50,000 PBMC/well of a 96-well U-bottomed plate plus 150,000 irradiated (2500 rad) PBMC/well as antigen-presenting cells (APCs) in 0.2 ml medium (RPMI 1640 with 1% human pooled AB serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 µg/ml penicillin G, and 100 [α] µg/ml streptomycin) for 5 days, followed by adding 5 ng/ml IL-2 (R & D Systems, Minneapolis, MN) twice per week. After three weeks, the culture plates were examined for cellular aggregation or "clump formation" by visual microscopy and the cells from the "best" 20-30 clump-forming wells among a total of 200 wells per each peptide Ag were expanded in 5 ng/ml IL-2 for another 1-2 weeks. These cells

were evaluated for peptide specificity by the proliferation assay, in which 50,000 T cells/well (washed 3x) were incubated in triplicate with 150,000 freshly isolated and irradiated APC/well plus either medium alone, 10 mg/ml MBP85-99 or 10 mg/ml CABL pep- tide for three days, with ³H-Tdy added for the last 18 hours. Stimulation index (S.I.) was calculated by dividing the mean CPM of peptide-added wells by the mean CPM of the medium alone control wells. T cell isolates with the highest S.I. for a particular peptide antigen were selected and expanded in medium containing 5 ng/ml IL-2, with survival of 1-6 months, depending on the clone, without further stimulations.